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Growth of *Bacteroides fragilis* in Rabbit Tracheal Organ Culture: Anaerobiosis and Tissue Respiration

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Rabbit tracheal explants supporting growth of inoculated *Bacteroides fragilis* in air were shown to keep low oxygen tension. Treating the explants with sodium azide induced high oxygen tension and arrested reversibly the growth of *B. fragilis*.

Bacteroides fragilis multiplied evidently shen point inoculated onto embedded rabbit tracheal culture and cultivated in air, but did not grow when inoculated onto inactivated explants (e.g., those treated by heating) and disappeared in a few days (8). It has been indicated for years that oxygen tension of environments and superoxide dismutase content of bacteria are most crucial for anaerobiosis (2, 7, 9, 11, 12). In the present investigation, the tracheal explants were measured for oxygen tension.

Embedded rabbit tracheal cultures were prepared as described previously (6). In brief, a tracheal fragment (2 by 5 by 1 mm thick) excised from an adult young rabbit was embedded, with the mucous surface upward, in 1.5 to 2.0% agar in L-15 medium (GIBCO Laboratories, Grand Island, N.Y.) in a plastic dish. The tracheal cultures thus prepared were kept in air at 37°C in a humidified box. The ciliary activity was observed under a microscope in reflected light.

B. fragilis strain NCTC 9343 propagated in agar-cooked meat medium (Difco Laboratories, Detroit, Mich.) was washed and suspended in phosphate-buffered saline (pH 7.2). With a platinum microloop (6), approximately 0.03 μl of the bacterial suspension was point inoculated onto the 1-day cultured mucous membrane. The mucous membrane incubated in air was homogenized at intervals and plated on GAM agar (Nissui Co., Tokyo) plates to examine for the viability and the increment of the bacteria. The plates were incubated for 2 days at 37°C in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.). The recovered bacteria were identified by the conventional slide agglutination technique with rabbit antiserum against *B. fragilis* NCTC 9343. Inability of the recovered bacteria to grow in air was also examined on GAM agar plates.

For measuring oxygen tension of inside tissues, a needle-type oxygen electrode is usually used; however, it is very difficult to apply it to such thin tissues as the explant. In addition, insertion of the electrode inevitably damages the tissue structure. Recently, a technique to measure blood oxygen through the skin has been developed (1, 3, 4). We applied such a transcutaneous oxygen electrode with some modification. A Nuclepore membrane filter pad (10-μm pore size, 10 μm thick; Nuclepore Corp., Pleasanton, Calif.) carrying an explant embedded in L-15 agar (0.4 mm thick) was placed on the wet electrode membrane of a transcutaneous oxygen electrode developed by Hagihara et al. (3) (PO-100, Sumitomo Electric Industrial Co., Osaka). Polyvinylidene chloride film (extremely low oxygen permeability, 12 μm thick; Kureha Kagaku, Tokyo) was chosen for use as the electrode membrane (Fig. 1). The explant was kept at 37°C on the electrode equipped with a thermister-controlled heater. Calibration was carried out with N_2 and air saturated with water vapor at 37°C. Oxygen tension of thin L-15 agar without the explant was 147.0 ± 1.0 mmHg (19.595 ± 0.133 kPa) (mean ± standard error). Measurements were performed in triplicate or more.

When a tracheal explant keeping a vigorous ciliary activity after cultivation for 1 day was placed on the oxygen electrode, oxygen tension decreased rapidly and reached a constant value of 3.1 ± 1.0 mmHg (0.413 ± 0.133 kPa) (Fig. 2). In contrast, the explant treated with ultraviolet light and that heat treated (8) showed oxygen tension of considerably high levels (63.0 ± 12.0 mmHg [8.398 ± 1.599 kPa] and 104.0 ± 9.0 mmHg [13.863 ± 1.199 kPa], respectively). Thus, the intact tracheal explant cultivated in air seems to provide a microenvironment of low oxygen tension, which is abolished by inactivation of the tissues.

Oxygen consumption by respiration of the tissue was thought to be the main factor for main-

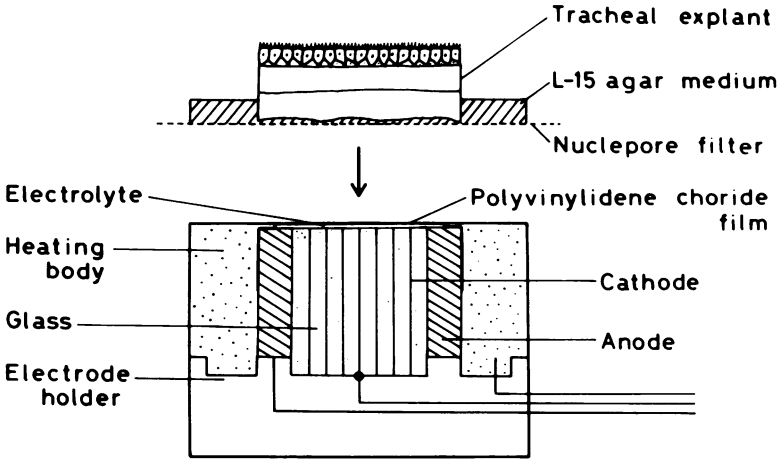

FIG. 1. *A schematic diagram of oxygen tension measurement. The Nuclepore filter carrying the explant was placed close to the electrode membrane with a detergent saline between them.*

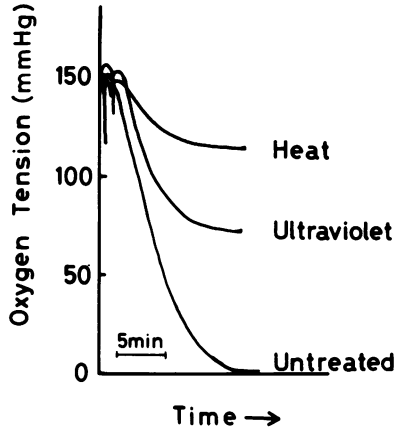

FIG. 2. *Tracing the oxygen tension of tracheal explants untreated and treated with ultraviolet light or heat measured with the transcutaneous oxygen electrode.*

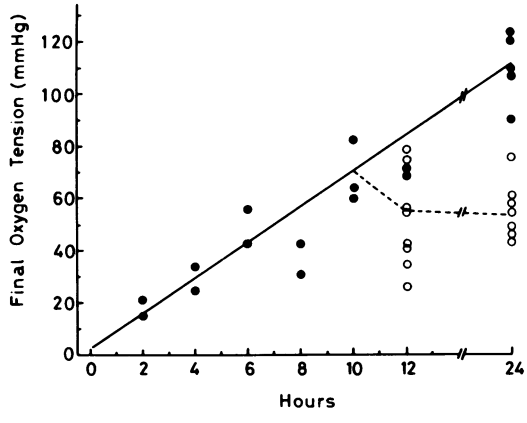

FIG. 3. *Oxygen tension of the tracheal explants exposed to sodium azide for various periods. Final oxygen tension of the explants treated with 1.0 mM sodium azide (●), final oxygen tension of the explants transplanted onto L-15 agar medium containing no sodium azide after a 10-h treatment with the drug* (○).

tenance of low oxygen tension. Sodium azide, an inhibitor of host cell respiration, was examined for its effect on the oxygen tension of the explant. Explants cultivated for 1 day were transplanted onto L-15 agar medium containing 1.0 mM sodium azide (Kanto Chemical Co., Tokyo), which did not affect the viability of *B. fragilis* in a GasPak jar, and they were assayed at intervals for oxygen tension. The oxygen tension of such explants increased gradually (Fig. 3). When the explants were transplanted within 10 h after the treatment onto L-15 agar medium containing no inhibitor, the ability to lower the oxygen tension was partially restored, resulting in 56.4 ± 4.1 mmHg (7.518 ± 0.546 kPa). Treatment for longer than 10 h, however, irreversibly damaged the explants. The ciliary activity of the explant diminished by treatment for 10 h was also restored after removal of the inhibitor (data not shown).

B. fragilis inoculated onto the untreated explants increased in population by 10^4-fold or more. As shown in Fig. 4, however, the bacteria failed to grow and disappeared in 72 h on the explants treated with 1.0 mM sodium azide from the beginning. When the treatment was started after 48 h of cultivation, the viable counts decreased after a 24-h lag. When the explant was treated with azide only for 10 h after 48 h of cultivation, the viable counts increased after removal of the inhibitor.

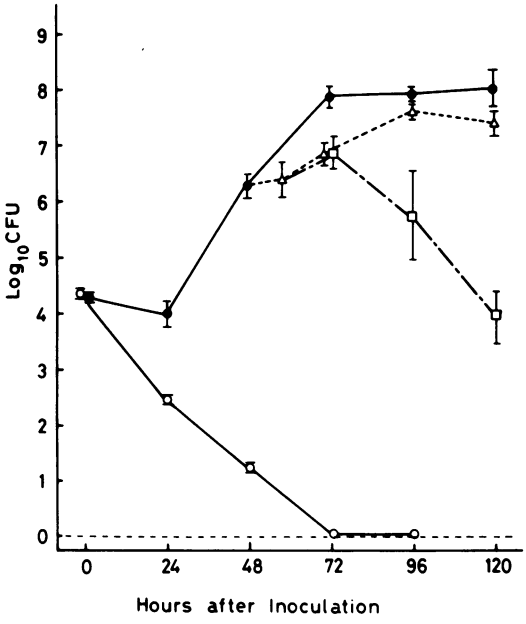

FIG. 4. *Viable bacteria in rabbit tracheal explants cultivated in air. B. fragilis was point inoculated onto each explant, and viable populations in the untreated explants (●) and in the explants treated with 1.0 mM sodium azide from the beginning (○) were counted at intervals. In 48 h after point inoculation, portions of untreated explants were transplanted onto L-15 agar medium containing sodium azide, and viable bacteria were counted (□). Some of these treated explants were transplanted again after 10 h onto L-15 agar medium containing no sodium azide, and viable bacteria in the explants were counted (△). Each point and bar represent the mean ± standard error of three to five measurements.*

Thus, the increased oxygen tension due to inhibition of respiration with sodium azide also halted the growth of *B. fragilis*. Since sodium azide is an inhibitor of ferrisuperoxide dismutase of *B. fragilis* (2, 11), its presence at such an increased oxygen tension may have enhanced the inhibition of the growth of the bacteria. Oxygen consumption by tissue respiration seems to produce an anaerobic microenvironment favorable for *B. fragilis*. Even under such conditions, the host cells can still utilize oxygen for respiration. It is well known that cytochrome oxidase of the tissue cells has a strong affinity to oxygen ($K_m < 1.0$ μM, 0.7 mmHg [0.093 kPa] at 25°C) (5). In in vivo tissues, oxygen tension is estimated to be about 40.0 mmHg (5.332 kPa) in capillaries and lower than 1.0 mmHg (0.133 kPa) in mitochondria. Therefore, there must be a gradient of the oxygen tension in tissue microenvironments. Many obligate anaerobes mysteriously inhabit in close proximity to the host cells requiring oxygen (13). We succeeded in culturing both the obligate anaerobe and aerobic host tissues under constant oxygen supply. Thus, in the vicinity of the respirating host cells, *B. fragilis* grows well, and its adhesive property to the host cells (10) might be beneficial for this kind of parasitism.

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